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(54) Title: MELANIN-CONCENTRATING HORMONE RECEPTOR

(57) Abstract: The present application features two different forms of the human MCH receptor: (1) MCH-R2 and (2) MCH-R3. Such MCH receptors provide a target for achieving a beneficial affect in a patient and facilitate the screening of compounds that modulate MCH receptor activity or expression. Beneficial effects that can be obtained include increasing appetite, decreasing appetite, and reducing stress.

TITLE OF THE INVENTION

MELANIN-CONCENTRATING HORMONE RECEPTOR

CROSS-REFERENCE TO RELATED APPLICATIONS

5   The present application claims priority to U.S. Serial No. 60/143,706, filed July 14, 1999, hereby incorporated by reference herein.

FIELD OF THE INVENTION

          The present invention relates to the melanin-concentrating hormone

10   receptor, methods of screening for compounds active at the melanin-concentrating hormone receptor, and methods of using such compounds to achieve a beneficial effect.

BACKGROUND OF THE INVENTION

15   The references cited herein are not admitted to be prior art to the claimed invention.

          Neuropeptides present in the hypothalamus play a major role in mediating the control of body weight. (Flier, et al., 1998. Cell, 92, 437-440.) Melanin-concentrating hormone (MCH) is a cyclic 19-amino acid neuropeptide

20   synthesized as part of a larger pre-prohormone precursor in the hypothalamus which also encodes neuropeptides NEI and NGE. (Nahon, et al., 1990. Mol. Endocrinol. 4, 632-637.) MCH was first identified in salmon pituitary, and in fish MCH affects melanin aggregation thus affecting skin pigmentation. In trout and in eels MCH has also been shown to be involved in stress induced or CRF–stimulated ACTH release.

25   (Kawauchi, et al., 1983. Nature 305, 321-323.)

          In humans two genes encoding MCH have been identified that are expressed in the brain. (Breton, et al., 1993. Mol. Brain Res. 18, 297-310.) In mammals MCH has been localized primarily to neuronal cell bodies of the hypothalamus which are implicated in the control of food intake, including perikarya

30   of the lateral hypothalamus and zona inertia. (Knigge, et al., 1996. Peptides 17, 1063-1073.)

          Pharmacological and genetic evidence suggest that the primary mode of MCH action is to promote feeding (orexigenic). MCH mRNA is up regulated in fasted mice and rats, in the ob/ob mouse (Qu, et al., 1996 Nature 380, 243-247), and

35   in mice with targeted disruption in the gene for neuropeptide Y (NPY). (Erickson, et

al., 1996. *Nature 381*, 415-418.) Injection of MCH centrally (ICV) stimulates food intake and MCH antagonizes the hypophagic effects seen with α melanocyte stimulating hormone (αMSH). (Qu, *et al.*, 1996. *Nature 380, 243-247.*) MCH deficient mice are lean, hypophagic and have increased metabolic rate. (Shimada, *et al.*, 1998. *Nature 396*, 670-673.)

MCH action is not limited to modulation of food intake as effects on the hypothalamic-pituitary-axis have been reported. (Nahon, 1994. *Critical Rev. in Neurobiol. 8*, 221-262.) MCH may be involved in the body response to stress as MCH can modulate the stress-induced release of CRF from the hypothalamus and ACTH from the pituitary. In addition, MCH neuronal systems may be involved in reproductive or maternal function.

SUMMARY OF THE INVENTION

The present application features two different forms of the human MCH receptor: (1) MCH-R2 and (2) MCH-R3. Such MCH receptors provide a target for achieving a beneficial affect in a patient and facilitate the screening of compounds that modulate MCH receptor activity or expression. Beneficial effects that can be obtained include increasing appetite, decreasing appetite, and reducing stress.

The MCH receptor is a G protein-coupled receptor that transduces an intracellular signal upon MCH binding. The ability of MCH-R1, a shorter length derivative of MCH-R2 and MCH-3 to couple to a G_i protein is illustrated in Example 3, *infra*.

MCH-R1, MCH-R2 and MCH-R3 are structurally related polypeptides differing by the presence of additional amino acids at the extracellular amino terminus. MCH-R2 has an additional 64 amino acids at its amino terminus compared to MCH-R1. MCH-R3 has an additional 5 amino acids at its amino terminus compared to MCHR-2, and an additional 69 amino acids at its amino terminus compared to MCHR-1. The nucleic acid and amino acid sequences of MCH-R1, MCH-R2, and MCH-R3 are provided for in SEQ. ID. NOs. 1, 2, 3, 4, 5, and 6 (see Example 1 *infra*). The additional nucleic acids and amino acid regions present in MCH-R3 compared to MCH-R1 is shown in SEQ. ID. NOs. 7 and 8.

Assays measuring MCH receptor activity can employ MCH-R3 or MCH-R2. MCH-R3 is believed to be a naturally occurring MCH receptor since it has the first in frame ATG start codon in the cDNA, while MCH-R1 and MCH-R2 appear

to be shorter-length versions of MCH-R3. MCH-R2 has the best ribosome initiation sequence 5' to the ATG start, 5 of the 9 nts match the optimal sequence: GCC GCC (A or G)CC ATG (SEQ. ID. NO. 10) which could result in MCH-R2 being the highest expressed form of the MCH receptor.

5   Thus, a first aspect of the present invention describes a purified nucleic acid comprising a nucleotide sequence encoding for at least 5 contiguous amino acids of SEQ. ID. NO. 8. In preferred embodiments the nucleotide sequence encodes for at least 9, at least 18, at least 27 or at least 36 contiguous amino acids of SEQ. ID. NO. 8; the nucleotide sequence encodes for amino acids 1-5 of SEQ. ID. NO. 8; the

10   nucleic acid comprises at least about 18, at least 27, or at least 54 contiguous nucleotides of SEQ. ID. NO. 7; and the nucleic acid comprises or consists of the nucleotide sequence of SEQ. ID. NO. 3, SEQ. ID. NO. 4, SEQ. ID. NO. 5, SEQ. ID. NO. 6, or SEQ. ID. NO. 9.

A "purified nucleic acid" represents at least 5% of the total nucleic

15   acid present in a sample or preparation. In preferred embodiments, the purified nucleic acid represents at least about 50%, at least about 75%, and at least about 95% of the total nucleic acid a sample or preparation. Reference to "purified nucleic acid" does not require that the nucleic acid have undergone any purification and may include, for example, chemically synthesized nucleic acid that has not been purified.

20   Another aspect of the present invention describes an expression vector able to express a polypeptide comprising at least 5 contiguous amino acids of SEQ. ID. NO. 8. The expression vector provides one or more regulatory elements functionally coupled to nucleic acid encoding for the polypeptide such that the polypeptide can be transcribed and translated when present in a suitable host.

25   Preferably, the expression vector contains an exogenous promoter transcriptionally coupled to the nucleic acid encoding the polypeptide.

In preferred embodiments the expression vector comprises nucleic acid encoding for at least 9, at least 18, at least 27 or at least 36 contiguous amino acids of SEQ. ID. NO. 8; the expression vector comprises nucleic acid encoding for amino

30   acids 1-5 of SEQ. ID. NO. 8; the expression vector comprises at least about 18, at least 27, or at least 54 contiguous nucleotides of SEQ. ID. NO. 7; and the expression vector comprises the nucleotide sequence of SEQ. ID. NO. 3, SEQ. ID. NO. 5, or SEQ. ID. NO. 9; and the expression vector comprises a nucleotide sequence encoding for polypeptide comprising or consisting of SEQ. ID. 4 or SEQ. ID. NO. 6.

Another aspect of the present invention describes a recombinant cell comprising an expression vector encoding for a polypeptide comprising at least 5 contiguous amino acids of SEQ. ID. NO. 8. The polypeptide can be expressed from the vector when present in the recombinant cell.

5   Another aspect of the present invention describes a method of preparing a MCH receptor polypeptide comprising the step of growing a recombinant cell containing an expression vector comprising nucleic acid encoding for a MCH receptor polypeptide under conditions suitable for the expression of the MCH receptor polypeptide. Preferably, the expressed MCH receptor polypeptide is purified.

10   Another aspect of the present invention describes a purified nucleic acid comprising a region of 20 contiguous nucleotides, wherein at least 16 nucleotides present in the region hybridizes to a complementary region of 20 contiguous nucleotides present in SEQ. ID. NO. 7 or the complement thereof. In preferred embodiments the nucleic acid comprises a region of 20 contiguous nucleotides

15   wherein at least 17, at least 18, at least 19, and 20, nucleotides present in the region hybridize to a complementary region of 20 contiguous nucleotides present in SEQ. ID. NO. 7 or the complement thereof.

Another aspect of the present invention describes a polypeptide comprising an amino acid sequence encoding for at least about 9 contiguous amino

20   acids of SEQ. ID. NO. 8, wherein the polypeptide is substantially free of associated proteins. In preferred embodiments the polypeptide comprises at least 18, at least 27, or at least 36 contiguous amino acids of SEQ. ID. NO. 8; and the polypeptide comprises or consists of the amino acid sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 6.

25   "Substantially free from associated proteins" means that the polypeptide is at least about 50%, preferably at least about 75%, and more preferably at least about 95% free from other cell membrane proteins which are normally found in a living mammalian cell expressing a MCH receptor.

Another aspect of the present invention describes a method for

30   screening for a compound able to bind a MCH receptor. The method involves the following: (a) expressing a polypeptide comprising MCH-R2, MCH-R3, or a fragment thereof, from recombinant nucleic acid, provided that the fragment comprises at least about 9 contiguous amino acids of SEQ. ID. NO. 8; (b) providing to the polypeptide a test preparation comprising one or more test compounds; and (c)

35   measuring the ability of the test preparation to bind to the polypeptide.

In different embodiments steps (b) and (c) are performed *in vitro*; steps (a), (b) and (c) are preformed using a whole cell; the polypeptide is expressed from an expression vector; the polypeptide comprises or consists of the amino sequence of SEQ. ID. NO. 6; and the method is performed where said step (b) further comprises
5   the presence of a labeled MCH, and step (c) measures the ability of said test preparation to inhibit binding of said labeled MCH to said polypeptide.

Another aspect of the present invention describes a method for screening for a compound able to modulate MCH receptor activity. The method involves (a) contacting a cell line expressing recombinant nucleic acid encoding for a
10   MCH receptor comprising or consisting of the amino acid sequence of MCH-R2 or MCH-R3 with a test preparation comprising one or more test compounds; and (b) measuring the effect of the test preparation on the activity of the receptor.

In preferred embodiments the MCH receptor comprises or consists of the amino acid sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 6; and the method
15   further comprises the presence of an MCH receptor agonist.

Another aspect of the present invention describes a method for suppressing appetite comprising the step of administering to a patient an effective amount of means for decreasing MCH receptor expression targeting a nucleic acid region within SEQ. ID. NO. 7. Such "means" are provided for by the materials and
20   structures described herein, and equivalents thereof. Preferred means are antisense nucleic acid and enzymatic nucleic acid. "Targeting a nucleic acid region within SEQ. ID. NO. 7" indicates that the means for decreasing MCH receptor activity contains a region substantially complementary to a segment of SEQ. ID. NO. 7 such that it hybridizes to the target under physiological conditions.

25   Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other
30   components and methodology useful for practicing the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present application describes a longer length version of MCH-R1 which is believed to be a natural MCH receptor (MCH-R3) and a slightly shorter
35   version thereof (MCH-R2). MCH-R2 and MCH-R3 have a variety of different uses

including being a target of receptor cloning, a target for receptor identification, a target for the production of antibodies, and a target for receptor modulation. Additional uses include being used in assays to determine the ability of a test preparation to modulate MCH receptor activity and in gene therapy.

5   Nucleic acid and amino acid sequences corresponding to MCH-R1 have been characterized in the art as encoding for a somatostatin-like receptor (SLC-1). For example, human and rat SLC-1 are described by Lakaye, *et al.*, 1998. *Biochimica et Biophysica ACTA* 1401:216-220 (which is not admitted to be prior art to the claimed invention). Additionally, a receptor characterized as a human

10   somatostatin-like receptor is referenced in International Publication No. WO 96/18651 and Kolakowski, *et al.*, 1996. *FEBS Letters 398*, 253-258, and an apparent splice variant is referenced in European Publication No. EP 0 848 060 A2 and International Publication No. WO 99/28492 (not admitted to be prior art to the claimed document).

15   A clone expressing the physiological correct receptor facilitates finding useful agonists or antagonists of the human MCH receptor. In contrast, use of a clone expressing a receptor with a physiologically incorrect MET start would lead to the use of an altered receptor protein, the use of which could be less predictive in finding compounds able to modulate MCH receptor activity.

20   The MCH receptor provides a target to achieve different beneficial effects in a patient. Preferably, MCH receptor activity is modulated to achieve one or more of the following: weight loss, weight gain, treat cancer (*e.g.*, colon or breast), reduce pain, treat diabetes, reduce stress, or teat sexual dysfunction.

             Modulation of MCH receptor activity can be achieved by evoking a

25   response at the MCH receptor or by altering a response evoked by an MCH receptor agonist or antagonist. Compounds modulating MCH receptor activity include agonists, antagonists, and allosteric modulators. Generally, MCH receptor antagonists and allosteric modulators negatively affecting activity will be used to achieve weight loss, treat cancer (*e.g.*, colon or breast), reduce pain, reduce stress, and/or teat sexual

30   dysfunction; and MCH receptor agonists and allosteric modulators positively affecting activity will be used to produce a weight gain.

             Preferably, MCH receptor activity is modulated to achieve a weight loss or to treat diabetes in a patient. Diabetes mellitus can be treated by modulating MCH receptor activity to achieve, for example, one or both of the following:

35   enhancing glucose tolerance or decreasing insulin resistance.

Excessive body weight is a contributing factor to different diseases, including hypertension, diabetes, dyslipidemias, cardiovascular disease, gall stones, osteoarthritis, and certain forms of cancers. Bringing about a weight loss can be used, for example, to reduce the likelihood of such diseases and as part of a treatment for

5 such diseases. Weight reduction can be achieved by modulating MCH receptor activity to obtain, for example, one or more of the following effects: reducing appetite, increasing metabolic rate, reducing fat intake, or reducing carbohydrate craving.

Facilitating a weight gain, maintenance in weight, or appetite increase

10 is particularly useful for a patient having a disease or disorder, or under going a treatment, accompanied by weight loss. Examples of diseases or disorders accompanied by weight loss include anorexia, bulimia, cancer cachexia, AIDS, wasting, cachexia, and wasting in frail elderly. Examples of treatments accompanied by weight loss include chemotherapy, radiation therapy, temporary or permanent

15 immobilization, and dialysis.

MCH RECEPTOR NUCLEIC ACID

The guidance provided in the present application can be used to obtain the MCH receptor from different sources such as mammalian sources and artificially

20 produced MCH receptor. Identification and isolation of MCH receptor nucleic acid is preferably performed using MCH-R3 nucleic acid information. Such nucleic acid information can be used to facilitate obtaining a full length receptor.

Obtaining nucleic acids encoding for related polypeptides is facilitated using sets of degenerative probes and primers and by the proper selection of

25 hybridization conditions. Sets of degenerative probes and primers are produced taking into account the degeneracy of the genetic code. Adjusting hybridization conditions is useful for controlling probe or primer specificity to allow for hybridization to nucleic acids having similar sequences.

Techniques employed for hybridization detection and PCR cloning are

30 well known in the art. Nucleic acid detection techniques are described, for example, in Sambrook, *et al.*, in *Molecular Cloning, A laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989. PCR cloning techniques are described, for example, in White, *Methods in Molecular Cloning*, volume 67, Humana Press, 1997.

MCH receptor probes and primers can be used to screen nucleic acid

35 libraries containing, for example, genomic DNA or cDNA. Such libraries are

commercially available, and can be produced using techniques such as those described in Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998. Detection of probe hybridization is facilitated through the use of a detectable label.

Starting with a MCH receptor obtained from a particular source,
5   derivatives can be produced having MCH receptor activity. Such derivatives include polypeptides having amino acid substitutions, additions and deletions. Such changes should be made outside of the MCH binding domain and in a manner not altering the tertiary structure. Amino acids are classified into certain types based on the structure of their R-groups. Substituting different amino acids within a particular group, such
10   as substituting valine for leucine, arginine for lysine, and asparagine for glutamine may not cause a change in functionality of the polypeptide.

Starting with a particular MCH receptor amino acid sequence and the known degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be obtained. The degeneracy of the genetic code arises because
15   almost all amino acids are encoded for by different combinations of nucleotide triplets. The translation of a particular codon into a particular amino acid is well known in the art (*see, e.g.*, Lewin *GENES IV*, p. 119, Oxford University Press, 1990).

Nucleic acid having a desired sequence can be synthesized using chemical and biochemical techniques. Examples of such techniques are described in
20   Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, and Sambrook, *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

Biochemical synthesis techniques involve the use of nucleic acid replicating conditions. Preferably, such techniques involve the use of a plasmid
25   containing MCH receptor nucleic acid and a compatible host cell. Examples of suitable techniques are provided by Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, and Sambrook, *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

Nucleic acid obtained from a particular source can be altered using
30   different techniques such as those provided for in Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, and Sambrook, *et al.*, in *Molecular Cloning, A laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

RECOMBINANT EXPRESSION

MCH receptor polypeptides such as a MCH receptor, a MCH receptor fragment, and a polypeptide containing the MCH receptor or MCH receptor fragment can be expressed from recombinant nucleic acid *in vivo* using a suitable host or *in vitro* using a translation system. Recombinantly expressed MCH receptor polypeptides are preferably used in assays to screen for compounds that bind to the MCH receptor and modulate the activity of the receptor.

Techniques for nucleic acid expression are well known in the art and can be applied to different nucleic acids encoding for different MCH receptor polypeptides. Examples of techniques for expression of nucleic acids are provided in Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, and Sambrook, *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

Preferably, expression is achieved in a host cell using an expression vector. An expression vector contains nucleic acid encoding for a desired polypeptide along with regulatory elements for proper transcription and processing. Generally, the regulatory elements that are present include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. Another preferred element is a polyadenylation signal providing for processing in eukaryotic cells. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids and viruses.

Preferably, an expression vector also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high copy number.

The skilled artisan can readily identify expression vectors providing suitable levels of MCH receptor polypeptide expression in different hosts. A variety of mammalian expression vectors are well known in the art including pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2), (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), pCI-neo (Promega) and .lambda.ZD35 (ATCC 37565). A variety of bacterial expression vectors are well known in the art including pET11a (Novagen), lambda gt11 (Invitrogen), pcDNAII (Invitrogen), and pKK223-3 (Pharmacia). A variety of fungal cell expression vectors are well known in the art including pYES2 (Invitrogen), Pichia expression vector (Invitrogen). A variety

of insect cell expression vectors are well known in the art including Blue Bac III
(Invitrogen).

Recombinant host cells may be prokaryotic or eukaryotic. Examples
of recombinant host cells include the following: bacteria such as *E. coli*; fungal cells
5   such as yeast; mammalian cells such as human, bovine, porcine, monkey and rodent;
and insect cells such as Drosophila and silkworm derived cell lines. Commercially
available mammalian cell lines include L cells L-M(TK.sup.-) (ATCC CCL 1.3), L
cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1
(ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1
10   (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC
CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC
CCL 171).

Expression vectors may be introduced into host cells using standard
techniques. Examples of such techniques include transformation, transfection,
15   lipofection, protoplast fusion, and electroporation.

MCH receptor nucleic acid can be expressed in a cell without the use
of an expression vector using, for example, synthetic mRNA or native mRNA.
Additionally, mRNA can be translated in various cell-free systems such as wheat
germ extracts and reticulocyte extracts, as well as in cell based systems, such as frog
20   oocytes. Introduction of mRNA into cell based systems can be performed, for
example, by microinjection.

MCH-R3 PROBES

Detection probes for MCH-R3 preferably contain a region targeted to a
25   SEQ. ID. NO. 7 nucleic acid region. The targeted region has at least 16 nucleotides
that hybridize (*e.g.*, A-T and G-C hybridization) to a complementary region of 20
contiguous nucleotides present in SEQ. ID. NO. 7 or the complement thereof. Such
probes can contain additional nucleic acid outside the targeted region to, for example,
provide for increased specificity or the serve another purpose such as being a reporter
30   sequence or a capture sequence.

Probes for the MCH receptor can specifically hybridize to MCH
receptor target nucleic acid under appropriate hybridization conditions (*i.e.*,
distinguish target nucleic acid from one or more non-target nucleic acid molecules).
Hybridization occurs through complementary nucleotide bases present on the probe or
35   primer and MCH receptor nucleic acid. Hybridization conditions determine whether

two molecules have sufficiently strong interactions with each other to form a stable hybrid.

Probes are composed of nucleic acids or derivatives thereof such as modified nucleic acid and peptide nucleic acid. Modified nucleic acid includes

5   nucleic acid with one or more altered sugar groups, altered internucleotide linkages, and/or altered nucleotide purine or pyrimidine bases. Detection of probe hybridization is facilitated through the use of a detectable label. Examples of detectable labels include luminescent, enzymatic, and radioactive labels. References describing modified nucleic acid include WO 98/02582, U.S. Patent No. 5,859,221 and U.S.

10   Patent No. 5,852,188, each of which are hereby incorporated by reference herein.

The degree of interaction between two molecules that hybridize together is reflected by the Tm of the produced hybrid. The higher the Tm the stronger the interactions and the more stable the hybrid. Tm is effected by numerous factors well known in the art such as the degree of complementarity, the type of

15   complementary bases present (*e.g.,* A-T hybridization versus G-C hybridization), and the structure of the nucleic acid backbones. *E.g.,* Sambrook, *et al.,* in *Molecular Cloning, A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

Stable hybrids are formed when the Tm of a hybrid is greater than the

20   temperature employed under a particular set of hybridization assay condition. The degree of specificity of a probe can be varied allowing for the identification of related sequences by adjusting the hybridization stringency conditions. Examples of stringency conditions are provided in Sambrook, *et al.,* in *Molecular Cloning, A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

25   An example of high stringency conditions is as follows: Prehybridization of filters containing DNA is carried out for 2 hours to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 μg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hours at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-

30   20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 minutes before autoradiography. Other procedures using conditions of high stringency would include, for example, either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48

hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

MCH-R2/MCH-R3 ANTIBODIES

5   Antibodies recognizing a MCH-R2 or MCH-R3 polypeptide can be produced using a SEQ. ID. NO. 8 polypeptide or a fragment thereof as an immunogen. Fragments of SEQ. ID. NO. 8 polypeptides used as an immunogen should be at least 9 amino acids in length. Antibodies to MCH-R3 can be used, for example, to identify and isolate MCH-R3 polypeptides. Examples of techniques for

10   producing and using antibodies are described in Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Harlow, *et al., Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, and Kohler, *et al., Nature 256*:495-497 (1975).

MCH RECEPTOR BINDING ASSAY

15   MCH-R2, MCH-R3, and fragments thereof, can be used in a binding assay to screen compounds able to bind to the MCH receptor. Different types of assay formats can be employed including the use of labeled compounds and/or the use of a labeled MCH ligand.

20   The particular amino acid sequence involved in MCH receptor binding can be readily identified by using labeled MCH and different receptor fragments. Different strategies can be employed to select fragments to be tested to narrow down the binding region. Examples of such strategies include testing consecutive fragments about 15 amino acids in length starting at the N-terminus, and testing longer length

25   fragments. If longer length fragments are tested, a fragment binding MCH can be subdivided to further locate the MCH binding region. Fragments used for binding studies can be generated using recombinant nucleic acid techniques.

          MCH ligands able to bind to the MCH receptor can readily be designed based on the structure of MCH and the ability of MCH derivatives to bind to the

30   MCH receptor. Examples of different polypeptides that appear to be MCH ligands are provided for in U.S. Patent No. 5,849,708, hereby incorporated by reference herein.

          Different types of labels for MCH ligands can be employed. Examples of such labels include radiolabels, luminescent molecules, haptens and enzyme

substrates. The ability of a particular label to interfere with binding can readily be determined by comparing the ability of MCH labeled with the particular label to compete against [^{125}I]-MCH binding.

5   Binding assays can be performed using individual compounds or preparations containing different numbers of compounds. A preparation containing different numbers of compounds having the ability to bind to the MCH receptor can be divided into smaller groups of compounds that can be tested to identify the compound(s) binding to the MCH receptor. In an embodiment of the present invention a test preparation containing at least 10 compounds is used in a binding

10   assay.

        Binding assays can be performed using recombinantly produced MCH receptor polypeptides present in different environments. Such environments include, for example, cell extracts, and purified cell extracts, containing the MCH receptor polypeptide expressed from recombinant nucleic acid; and also include, for example,

15   the use of a purified MCH receptor polypeptide produced by recombinant means which is introduced into a different environment.

SCREENING FOR MCH RECEPTOR ACTIVE COMPOUNDS

        Screening for MCH receptor active compounds is facilitated using

20   recombinantly expressed MCH-R2, MCH-R3 or a chimeric receptor containing a fragment thereof functionally coupled to a G protein. Using such recombinantly expressed MCH receptor polypeptides offers several advantages such as the ability to express the receptor in a defined cell system so that a response to MCH receptor active compounds can more readily be differentiated from responses to other

25   receptors. For example, the MCH receptor can be expressed in a cell line such as HEK 293, COS 7, and CHO not normally expressing the receptor by an expression vector, wherein the same cell line without the expression vector or with an expression vector not encoding for a MCH receptor can act as a control.

        Screening for MCH receptor active compounds is facilitated through

30   the use of a MCH ligand in the assay. The use of a MCH ligand in a screening assay provides for MCH receptor activity. The effect of test compounds on such activity can be measured to identify, for example, allosteric modulators and antagonists. Additionally, such assays can be used to identify agonists.

        MCH receptor activity can be measured using different techniques

35   such as detecting a change in the intracellular conformation of the MCH receptor, G

protein activities, and/or intracellular messengers. G protein activities include Gi and Gs. Gi activity can be measured using techniques well known in the art such as a melonaphore assay, assays measuring cAMP production, inhibition of cAMP accumulation, and binding of ^{35}S-GTP. cAMP can be measured using different

5   techniques such as radioimmunoassay and indirectly by cAMP responsive gene reporter proteins.

MCH receptor activity can be measured, for example, by assays measuring the phospholipase C signal transduction pathway. Activity of the phospholipase C signal transduction pathway can be measured using standard

10 techniques such as those measuring intracellular Ca^{2+}. Examples of techniques well known in the art that can be employed to measure Ca^{2+} include the use of dyes such as Fura-2 and the use of Ca^{2+}-bioluminescent sensitive reporter proteins such as aequorin. An example of a cell line employing aequorin to measure G protein activity is HEK293/aeq17. (Button *et al.*, 1993. *Cell Calcium 14*, 663-671, and

15 Feighner *et al.*, 1999. *Science 284*:2184-2188, both of which are hereby incorporated by reference herein.)

Chimeric receptors containing one or more MCH receptor regions functionally coupled to polypeptides from other G proteins can also be used to measure activity. A chimeric MCH receptor contains an N-terminal extracellular

20 domain; a transmembrane domain made up of transmembrane regions, extracellular loop regions, and intracellular loop regions; and an intracellular carboxy terminus domain.

The specificity of G protein coupling is determined by intracellular domain(s). A chimeric G protein coupled receptor can be produced to functionally

25 couple to a particular G protein such as a Gq protein or a Gi protein. Such signal swapping allows for the detection of a receptor activity by measuring Gq or Gi activity. Techniques for producing chimeric receptors and measuring G protein coupled responses are provided for in, for example, International Application No. WO 97/05252, and U.S. Patent No. 5,264,565, both of which are hereby incorporated by

30 reference herein.

Functional assays can be performed using individual compounds or preparations containing different compounds. A preparation containing different compounds where one or more compounds affect MCH receptor or chimeric receptor activity can be divided into smaller groups of compounds to identify the compound(s)

affecting MCH receptor activity. In an embodiment of the present invention a test preparation containing at least 10 compounds is used in a functional assay.

Functional assays can be performed using recombinantly produced MCH receptor polypeptides or chimeric receptor polypeptides present in different

5   environments. Such environments include, for example, cell extracts, and purified cell extracts, containing the MCH receptor polypeptide expressed from recombinant nucleic acid; and the use of a purified MCH receptor polypeptide produced by recombinant means that is introduced into a different environment.

Preferably, recombinantly expressed MCH receptor polypeptide is

10   expressed from an expression vector. More preferably, the recombinantly expressed MCH receptor polypeptide comprises or consists of an amino acid sequence provided for in SEQ. ID. NOs. 4 or 6.

MODULATING MCH RECEPTOR EXPRESSION

15   MCH receptor expression can be altered as a means for increasing or decreasing MCH receptor activity. Such alterations include inhibiting MCH receptor nucleic acid activity to reduce MCH receptor expression and supplying MCH receptor nucleic acid to increase MCH receptor activity.

20   Inhibition of MCH Receptor Nucleic Acid Activity

MCH receptor nucleic acid activity can be inhibited using nucleic acids recognizing MCH receptor nucleic acid and affecting the ability of such nucleic acid to be transcribed or translated. Inhibition of MCH receptor nucleic acid activity can be used, for example, in target validation studies looking at appetite and stress in

25   model systems, and to inhibit appetite or stress.

A preferred target for inhibiting MCH receptor translation is mRNA. The ability of mRNA to be translated into a protein can be effected by compounds such as anti-sense nucleic acid and enzymatic nucleic acid.

Anti-sense nucleic acid can hybridize to a region of a target mRNA.

30   Depending on the structure of the anti-sense nucleic acid, anti-sense activity can be brought about by different mechanisms such as blocking the initiation of translation, preventing processing of mRNA, hybrid arrest, and degradation of mRNA by RNAse H activity.

Enzymatic nucleic acid can recognize and cleave another nucleic acid

35   molecule. Preferred enzymatic nucleic acids are ribozymes.

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General structures for anti-sense nucleic acids and ribozymes and methods of delivering such molecules are well known in the art. Modified and unmodified nucleic acids can be used to exert anti-sense effects. Different types of modifications can effect certain anti-sense activities such as the ability to be cleaved

5   by RNAse H, and can effect nucleic acid stability. Examples of references describing different anti-sense molecules and ribozymes, and the use of such molecules are provided in U.S. Patent Nos. 5,849,902, 5,859,221, and 5,852,188, which are each hereby incorporated by reference herein.

Guidelines for pharmaceutical administration in general are provided

10   in, for example, *Remington's Pharmaceutical Sciences 18th Edition*, Ed. Gennaro, Mack Publishing, 1990, and *Modern Pharmaceutics 2nd Edition*, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990. Nucleic acid can be introduced into cells present in different environments using *in vitro*, *in vivo*, or *ex vivo* techniques.

15   ### Increasing MCH Receptor Expression

Nucleic acid coding for the MCH receptor can be used, for example, to cause an increase in appetite and to create a test system (*e.g.*, a transgenic animal) for screening for compounds affecting MCH receptor expression. Nucleic acids can be introduced and expressed in cells present in different environments. Guidelines for

20   pharmaceutical administration in general are provided in, for example, *Remington's Pharmaceutical Sciences 18th Edition*, *supra*. and *Modern Pharmaceutics 2nd Edition*, *supra*. Examples of techniques useful in gene therapy are illustrated in *Gene Therapy & Molecular Biology: From Basic Mechanisms to Clinical Applications*, Ed. Boulikas, Gene Therapy Press, 1998 (hereby incorporated by reference herein).

25

MODULATING MCH RECEPTOR ACTIVITY

Using the present application as a guide compounds able to modulate MCH receptor activity can be obtained and used to achieve a beneficial effect in a patient. Such effects can be achieved, for example, by altering appetite or relieving

30   stress using a compound active at the MCH receptor.

Altering appetite is particularly useful for gaining weight in an under weight patient or losing weight in an over weight patient. In addition, for example, farm animals can be treated to gain weight. Under weight patients include those having a body weight about 10% or less, 20% or less, and 30% or less, than the lower

35   end of a "normal" weight range or Body Mass Index ("BMI"). Over weight patients

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include those having a body weight about 10% or more, 20% or more, 30% or more, or 50% or more, than the upper end of a "normal" weight range or BMI. "Normal" weight ranges are well known in the art and take into account factors such as a patient age, height, and body type.

5   BMI measures your height/weight ratio. It is determined by calculating weight in kilograms divided by the square of height in meters. The BMI "normal" range is 19-22.

Preferably, non-protein MCH receptor antagonists are used to alter MCH receptor activity. Such antagonists are preferably organic compounds 10 comprising one or more aryl or heteroaryl and having a molecule weight between about 150 and 900.

MCH receptor antagonists include compounds binding to the MCH receptor binding site and compounds binding at other sites. Such compounds can be identified using the techniques described herein. Preferably, an MCH receptor 15 antagonist binds with an affinity of at least about 0.001-fold as well [^{125}I]-MCH using the *in vitro* MCH binding assay, or has an IC_{50} of at least 5 μM as determined by the *in vitro* MCH binding assay or the melanophore assay (using *e.g.*, MCH at a concentration of 150 nM in 0.1N acetic acid and MCH-R3). In additional embodiments, the antagonist binds at least 0.01-fold, or at least 0.1-fold, as well as 20 [^{125}I]-MCH, or 0.1-fold to 0.05-fold as well as [^{125}I]-MCH using the *in vitro* MCH binding assay; and the MCH receptor antagonist has an IC_{50} of at least 500 nM.

MCH receptor modulating compounds can be provided in kit. Such a kit typically contains an active compound in dosage forms for administration. A dosage form contains a sufficient amount of active compound such that a beneficial 25 effect can be obtained when administered to a patient during regular intervals, such as 1 to 6 times a day, during the course of 1 or more days.

Preferred kits contain a MCH receptor antagonist provided in dosage forms, wherein the antagonist binds with an affinity of at least about 0.001-fold as well as [^{125}I]-MCH using the *in vitro* MCH binding assay or has an IC_{50} of at least 30 about 5 μM. More preferably, the kits contain instructions indicating the use of the dosage form for weight reduction (*e.g.*, obesity or overweight) or stress reduction, and the amount of dosage form to be taken over a specified time period.

DOSING FOR THERAPEUTIC APPLICATIONS

Guidelines for pharmaceutical administration in general are provided in, for example, *Remington's Pharmaceutical Sciences 18th Edition*, Ed. Gennaro, Mack Publishing, 1990, and *Modern Pharmaceutics 2nd Edition*, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990, both of which are hereby incorporated by reference herein.

Compounds activity active at the MCH receptor having appropriate functional groups can be prepared as acidic or base salts. Pharmaceutically acceptable salts (in the form of water- or oil-soluble or dispersible products) include conventional non-toxic salts or the quaternary ammonium salts that are formed, *e.g.*, from inorganic or organic acids or bases. Examples of such salts include acid addition salts such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate; and base salts such as ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine.

MCH receptor active compounds can be administered using different routes including oral, nasal, by injection, transdermal, and transmucosally. Active ingredients to be administered orally as a suspension can be prepared according to techniques well known in the art of pharmaceutical formulation and may contain microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents. As immediate release tablets, these compositions may contain microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants.

When administered by nasal aerosol or inhalation, compositions can be prepared according to techniques well known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable

preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents.

The compounds may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or
5   intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. When administered by injection, the injectable solutions or suspensions may be formulated using suitable non-toxic, parenterally-acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending
10   agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

When rectally administered in the form of suppositories, compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at
15   ordinary temperatures, but liquidify and/or dissolve in the rectal cavity to release the drug.

Suitable dosing regimens for the therapeutic applications of the present invention are selected taking into factors well known in the art including age, weight, sex and medical condition of the patient; the severity of the condition to be treated;
20   the route of administration; the renal and hepatic function of the patient; and the particular compound employed. Guidelines for pharmaceutical administration including pharmaceutical compositions are provided in, for example, *Remington's Pharmaceutical Sciences 18th Edition, supra.* and *Modern Pharmaceutics 2nd Edition, supra.*

25   Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug. The daily dose for a patient is expected to be between 0.01 and 1,000 mg per adult patient per day.

30

EXAMPLES

Examples are provided below to further illustrate different features and advantages of the present invention. The examples also illustrate useful methodology
35   for practicing the invention. These examples do not limit the claimed invention.

Example 1: MCH Receptor Related Sequences

Human MCH receptor related sequences are provided as follows:

5   Human MCH-R1 Nucleic Acid Sequence (SEQ. ID. NO. 1)
     ATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAACGCCAGCAACAC
     CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
     GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT
     GCCTCCTGGGCATCATCGGGAACTCCACGGTCATCTTCGCGGTCGTGAAG
10   AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA
     CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA
     CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC
     TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG
     ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC
15   ACGAAGTTCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGCCTCCTGTGG
     GCCCTCTCCTTCATCAGCATCACCCCTGTGTGGCTGTATGCCAGACTCATC
     CCCTTCCCAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCCCAACCCAGA
     CACTGACCTCTACTGGTTCACCCTGTACCAGTTTTTCCTGGCCTTTGCCCTG
     CCTTTTGTGGTCATCACAGCCGCATACGTGAGGATCCTGCAGCGCATGAC
20   GTCCTCAGTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGCGGACAAAGA
     GGGTGACCCGCACAGCCATCGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG
     CACCCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGCCGCCCGACCC
     TCACCTTTGTCTACTTATACAATGCGGCCATCAGCTTGGGCTATGCCAACA
     GCTGCCTCAACCCCTTTGTGTACATCGTGCTCTGTGAGACGTTCCGCAAAC
25   GCTTGGTCCTGTCGGTGAAGCCTGCAGCCCAGGGGCAGCTTCGCGCTGTC
     AGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGCAAAGGCACCT
     GA

     Human MCH-R1 Amino Acid Sequence (SEQ. ID. NO. 2)
30   MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSISYINIIMPSVFGTICLLGIIG
     NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH
     FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSVATLVI
     CLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLAFA
     LPFVVITAAYVRILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY

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YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV
KPAAQGQLRAVSNAQTADEERTESKGT

Human MCH-R2 Nucleic Acid Sequence (SEQ. ID. NO. 3)

ATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTGGAGGCGGCAGCGGCT
GCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGCGGGGCTTGCGCTCCG
GGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGCCTGCGTGGGTGGAGG
GGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGGCACTGGCTGGATGGAC
CTGGAAGCCTCGCTGCTGCCCACTGGTCCCAACGCCAGCAACACCTCTGA
TGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGGGGAGCA
TCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCTGCCTCC
TGGGCATCATCGGGAACTCCACGGTCATCTTCGCGGTCGTGAAGAAGTCC
AAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAACCTCTCG
GTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCACCAGCTC
ATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCCTCATCAC
GGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTGACCGCCA
TGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCCACGAAGT
TCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGCCTCCTGTGGGCCCTCT
CCTTCATCAGCATCACCCCTGTGTGGCTGTATGCCAGACTCATCCCCTTCC
CAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCCCAACCCAGACACTGAC
CTCTACTGGTTCACCCTGTACCAGTTTTTCCTGGCCTTTGCCCTGCCTTTTG
TGGTCATCACAGCCGCATACGTGAGGATCCTGCAGCGCATGACGTCCTCA
GTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGCGGACAAAGAGGGTGAC
CCGCACAGCCATCGCCATCTGTCTGGTCTTCTTTGTGCTGGGCACCCTA
CTATGTGCTACAGCTGACCCAGTTGTCCATCAGCCGCCCGACCCTCACCTT
TGTCTACTTATACAATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCT
CAACCCCTTTGTGTACATCGTGCTCTGTGAGACGTTCCGCAAACGCTTGGT
CCTGTCGGTGAAGCCTGCAGCCCAGGGGCAGCTTCGCGCTGTCAGCAACG
CTCAGACGGCTGACGAGGAGAGGACAGAAAGCAAAGGCACCTGA

Human MCH-R2 Amino Acid Sequence (SEQ. ID. NO. 4)
MKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGGRRWRLPQPAWVEG
SSARLWEQATGTGWMDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSISYI
NIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLG
MPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVH

PISSTKFRKPSVATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDT
DLYWFTLYQFFLAFALPFVVITAAYVRILQRMTSSVAPASQRSIRLRTKRVTRT
AIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYI
VLCETFRKRLVLSVKPAAQGQLRAVSNAQTADEERTESKGT

5

Human MCH-R3 Nucleic Acid Sequence (SEQ. ID. NO. 5)
ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG
GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC
GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC
10 CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAACG
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT
CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTC
GGCACCATCTGCCTCCTGGGCATCATCGGGAACTCCACGGTCATCTTCGCG
15 GTCGTGAAGAAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTT
CATCATCAACCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTT
CATGATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCA
TGTGCACCCTCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACC
TACATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCC
20 ATCTCTTCCACGAAGTTCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGC
CTCCTGTGGGCCCTCTCCTTCATCAGCATCACCCCTGTGTGGCTGTATGCC
AGACTCATCCCCTTCCCAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCC
CAACCCAGACACTGACCTCTACTGGTTCACCCTGTACCAGTTTTTCCTGGC
CTTTGCCCTGCCTTTTGTGGTCATCACAGCCGCATACGTGAGGATCCTGCA
25 GCGCATGACGTCCTCAGTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGC
GGACAAAGAGGGTGACCCGCACAGCCATCGCCATCTGTCTGGTCTTCTTT
GTGTGCTGGGCACCCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGC
CGCCCGACCCTCACCTTTGTCTACTTATACAATGCGGCCATCAGCTTGGGC
TATGCCAACAGCTGCCTCAACCCCTTTGTGTACATCGTGCTCTGTGAGACG
30 TTCCGCAAACGCTTGGTCCTGTCGGTGAAGCCTGCAGCCCAGGGGCAGCT
TCGCGCTGTCAGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGC
AAAGGCACCTGA

Human MCH-R3 Amino Acid Sequence (SEQ. ID. NO. 6)

MSVGAMKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGGRRWRLPQP
AWVEGSSARLWEQATGTGWMDLEASLLPTGPNASNTSDGPDNLTSAGSPPR
TGSISYINIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD
5   LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMAIDR
YLATVHPISSTKFRKPSVATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGI
RLPNPDTDLYWFTLYQFFLAFALPFVVITAAYVRILQRMTSSVAPASQRSIRLR
TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS
CLNPFVYIVLCETFRKRLVLSVKPAAQGQLRAVSNAQTADEERTESKGT

10

SEQ. ID. NO. 7

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG
GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC
GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC
15 CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG
CACTGGCTGG

SEQ. ID. NO. 8

MSVGAMKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGGRRWRLPQP
20 AWVEGSSARLWEQATGTGW

MCH-R3 DNA with Intron (lower case) SEQ. ID. NO. 9

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG
GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC
25 GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC
CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAACG
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGgtgagttgactggg
agccctccctcctctgggctgtgggtggaaaatgggaaggtttcacccctgagccaaactgcttgggaaactttatcacagtt
30 cttggggacaagatctgtggtctgctttgctctgaggggcaggagaaaagggggcaatggtccgcaggggcagacgggc
aggagcagagcaggggcgaaggcatattcagaatggcaaggaaggggggccagccgtgagacagcaggggaaggc
tcgctgctgggttccaaagatgcttggcagaaaaaattccaggctggaaaagcaagcgagagaagctggagggtggtatgt
gggagacagctggggggctcactcctgcactgttagcctcagctttttactcccacttggatgatgaggtctgagacatccttac
tgccacctgggagaggccctgggaagggaagacttcacagagccatgaggggattaacttttctggtgaattaagcttcctg
35 acatttccagagctgcggtgccctgggattccagctttgaaggagaaaggaaggaaggaaaagaggaaaggcttatgtag

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ataattttttccaggctgctgagctccaacagacagtttctgtctctgcttcactcaagaagcccaggctcagaagataccaatc

aaggaaatccccgctaggaagcctggggtagggagagctgctggcttgaccagggcacagccggcaaaagcctctacaa

gacagtcacccacagatatgcccaagaatcagtacacagtttccaaccagagatctccaaaatgaaacactcagggctaca

cataggaaaagcacgcacacacacacacacacacatacacagacacttacttttgtgtccttctggctatgctgacgagttttc

5   ctggtgaagcccggggctcacagagtaatctctgcagacaactgtggttcttgcctctggtgcctgcaggaggcaggcatgt

tgtgtccttccaagacagatggctcagggcactctggtaggattcaccaggaaactcatggagaagggaaaagggacaag

attagcaacagtgaagggagggagaatggtgggagaggattccagatgaacggtgggtcgctggaggctgagcatgcca

gcaggatgtcagttctcagagcaaagcccatgtcaaacagccaacgcttgctccttctgtccccagGATCACCTCC

TCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGG

10   CACCATCTGCCTCCTGGGCATCATCGGGAACTCCACGGTCATCTTCGCGGT

CGTGAAGAAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCA

TCATCAACCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCAT

GATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGT

GCACCCTCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTAC

15   ATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATC

TCTTCCACGAAGTTCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGCCTC

CTGTGGGCCCTCTCCTTCATCAGCATCACCCCTGTGTGGCTGTATGCCAGA

CTCATCCCCTTCCCAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCCCAA

CCCAGACACTGACCTCTACTGGTTCACCCTGTACCAGTTTTTCCTGGCCTT

20   TGCCCTGCCTTTTGTGGTCATCACAGCCGCATACGTGAGGATCCTGCAGCG

CATGACGTCCTCAGTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGCGGA

CAAAGAGGGTGACCCGCACAGCCATCGCCATCTGTCTGGTCTTCTTTGTGT

GCTGGGCACCCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGCCGCC

CGACCCTCACCTTTGTCTACTTATACAATGCGGCCATCAGCTTGGGCTATG

25   CCAACAGCTGCCTCAACCCCTTTGTGTACATCGTGCTCTGTGAGACGTTCC

GCAAACGCTTGGTCCTGTCGGTGAAGCCTGCAGCCCAGGGGCAGCTTCGC

GCTGTCAGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGCAAAG

GCACCTGA

30   Example 2: Melanophore Assay For Determination

Of G protein-Coupled Receptor Activation

          Melanophores were transfected with either a vector designed to

synthesize SLC-1 mRNA and thereby lead to overexpression of SLC-1 receptor (i.e.,

35   pcDNA3-hSLC-1), the empty vector (pcDNA3), and plasmids encoding control

receptors (*i.e.*, pcDNA1amp-CB2 and pcDNA3-thromboxane A2). The plasmid
vector pcDNA3 (Invitrogen) was used to express the recombinant SLC-1 mRNA and
protein in cells transfected with this construct. The coding sequence of the SLC-1
cDNA without its untranslated 5' and 3' sequences (SEQ. ID. NO. 1) was subcloned

5   by blunt-end ligation into the EcoRV site of pcDNA3. The SLC-1 cDNA insert can
be excised from pcDNA3-hSLC-1 by restriction digestion of the plasmid with KpnI
and XbaI. The amino-terminal coding end of the SLC-1 insert was cloned proximal to
the Cytomegalovirus promoter contained in pcDNA3. The carboxyl-terminal coding
sequences of the SLC-1 insert was cloned proximal to the bovine growth hormone

10   polyadenylation signal sequence in pcDNA3.

Growth of *Xenopus laevis* melanophores and fibroblasts was
performed as described previously (Daniolos, *et al.*, 1992. *Pigment Cell Res.* 3, 38-
43; and Lerner, 1994. *Trends Neurol. Sci.* 17, 142-146). Briefly, melanophores were
grown in *Xenopus* fibroblast-conditioned growth medium. The fibroblast-conditioned

15   growth medium was prepared by growing *Xenopus* fibroblasts in 70% L-15 medium
(Sigma), pH 7.3, supplemented with 20 % heat inactivated fetal bovine serum (Life
Technologies, Mississauga, Ont), 100 μg/ml streptomycin, 100 units/ml penicillin and
2 mM glutamine at $27^{o}C$. The medium from growing fibroblasts was collected,
passed through a 0.2 micron filter (referred to as fibroblast-conditioned growth

20   medium) and used to culture melanophores at $27^{o}C$. Plasmid DNA was transiently
transfected into melanophores by electroporation using a BTX ECM600
electroporator (Genetronics, Inc. San Diego, CA).

Melanophores were incubated in the presence of fresh fibroblast-
conditioned growth medium for 1 hour prior to harvesting of cells. Melanophore

25   monolayers were detached by trypsinization (0.25% trypsin, JHR Biosciences,
Lenexa, KS), followed by inactivation of the trypsin with fibroblast-conditioned
growth medium.

The cells were collected by centrifugation at 200 x *g* for 5 minutes at
$4^{o}C$. Cells were washed once in fibroblast conditioned growth medium, centrifuged

30   (200 x g, 5 minutes, 4°C) and resuspended at 5 x 10^{6} cells per ml in ice cold 70%
PBS pH 7.0. 400 μl aliquots of cells in PBS were added to prechilled 1.5 ml tubes
containing 2 μg of pcDNA3-hSLC-1 plasmid DNA, 2 μg each of two internal control
GPCRs (pcDNA1amp-cannabinoid 2 and pcDNA3-thromboxane A2; Slipitz, *et al.*,
1995. *Mol. Pharmacol.* 48, 352-361), and 18 μg of pcDNA3.1 plasmid vector DNA

for a total of 24 μg DNA in a 40 μl total volume. Samples were incubated on ice for 20 minutes with mixing every 7 minutes.

Cell and DNA mixes were transferred to prechilled 0.2 mm gap electroporation cuvettes (BTX) and electroporated using the following settings:

5   capacitance of 325 microfarad, voltage of 450 volts and resistance of 720 ohms. Immediately following electroporation, cells were mixed with fibroblast-conditioned growth medium and plated onto flat bottom 96 well microtiter plates (NUNC). Electroporations from multiple cuvettes were pooled together prior to plating to ensure homogenous transfection efficiency.

10   On the day following transfection, the media was replaced with fresh fibroblast-conditioned growth media and incubated for one to three days at 27°C prior to assaying for receptor expression. On the day of ligand stimulation, medium was removed by aspiration and cells were washed with 70% L-15 media containing 15 mM HEPES, pH 7.3.

15   Assays were divided into two separate parts in order to examine Gs/Gq-coupling which results in pigment dispersion in melanophores, or Gi-coupling which results in pigment aggregation. For Gs/Gq-coupling responses, assays were performed as follows. Cells were incubated in 100 μl of 70% L-15 media containing 15 mM HEPES, pH 7.3, for 1 hour in the dark at room temperature, and then

20   incubated in the presence of melatonin (2 nM final concentration) for 1 hour in the dark at room temperature to induce pigment aggregation. Initial absorbance readings at 600 nm were measured using a Bio-Tek Elx800 Microplate reader (ESBE Scientific) prior to addition of ligand. Ligands (100 nM final concentration in DMSO for small molecules non-peptides or 150 nM final concentration in 0.1N acetic acid

25   for peptide molecules) were added to wells, mixed, and incubated in the dark at room temperature for 1 hour, after which the final absorbance at 600 nm was determined.

For Gi-coupled responses, cell monolayers plated in 96-well microtiter plates were incubated in the presence of 100 μl/well of 70% L-15 media containing 2% fibroblast-conditioned growth medium, 2 mM glutamine, 100 μg /ml

30   streptomycin, 100 units/ml penicillin and 15 mM HEPES, pH 7.3, for 15 minutes in the dark at room temperature to preset the cells to dispersion. Initial absorbance readings at 600 nm were determined, followed by the addition of ligands. After a 1.5 hour incubation in the dark at room temperature final absorbances were determined. Absorbance readings were converted to transmission values to quantitate pigment

dispersion using the following formula: 1- Tf/Ti, where Ti = the initial transmission at 600 nm and Tf = the final transmission at 600 nm.

Example 3: MCH-R1 Receptor Activity

5

The *Xenopus* melanophore system (Daniolos, *et al.*, 1992. *Pigment Cell Res.* 3, 38-43) is based on the dispersion and aggregation of intracellular pigment granules in response to changes in intracellular second messenger molecules. Agonist activation of a recombinant Gs- or Gq-coupled receptor expressed heterologously in

10   melanophores leads to pigment dispersion. Conversely, agonist activation of a recombinant Gi-coupled receptor expressed heterologously in melanophores leads to pigment aggregation. Changes in the melanophore pigmentation show a dose-dependent correlation with the level of specific receptor activation, and can be quantified by the change in absorbance at 600 nm between the nonactivated and

15   agonist-activated cells (Daniolos, *et al.*, 1992. *Pigment Cell Res.* 3, 38-43).

Melanophores transiently transfected with plasmid DNAs expressing "SLC-1", cannabinoid 2 and thromboxane A2 receptors were plated onto 96 well microtiter plates. Following the above pretreatment conditions, cells were incubated for 1 hour in the presence of a collection of 202 known small molecules and peptides

20   including MCH. The test ligand collection included 80 small molecules (a different test molecule in each well) at 100 nM final concentration, 80 peptides at 150 nM final concentration and 42 small molecules and peptides at 500 and 1000 nM final concentration, respectively.

Pigment aggregation responses (Gi-coupled responses) were detected

25   with the following five peptides from the peptide plate (150 nM final concentration): thrombin, MCH, valosin, RANTES and CGRP with responses ranging from 33% to 66% of the positive cannabinoid 2 receptor control activated by the cannabinoid receptor agonist, HU-210. Background aggregation responses in this assay range from 0 – 25% of the positive control cannabinoid 2 receptor response. The response

30   seen for thrombin is detected in mock (non-receptor) transfected melanophores and represents activation of the endogenous melanophore thrombin receptor. Other controls include a positive aggregation response to melatonin, stimulating the endogenous Xenopus receptor and a lack of aggregation response to PBS, acetic acid or DMSO vehicle controls (15%, 11% and 3% of the positive cannabinoid 2 receptor

35   positive control value, respectively).

Example 4: MCH Receptor Binding Assay

The MCH receptor binding assay can be conducted on cells transfected with a MCH-R2 expression plasmid (full-length open reading frame of SEQ. ID. NO. 5 placed in the mammalian expression vector pcDNA-3 (Invitrogen, Carlsbad, CA) or a MCH-R2 expression plasmid (full-length open reading frame of SEQ. ID. NO. 3 placed in the mammalian expression vector pcDNA-3 (Invitrogen, Carlsbad, CA) to produce a MCH-R expression plasmid. Mammalian cells HEK-293 or COS-7 are transfected with vector using Lipofectamine (GIBCO-BRL; Hawley-Nelson, P. 1993, *Focus* 15:73). Transfections are performed in 60 mm dishes on 80% confluent cells (approximately 4 x 10^5 cells) with 8 μg of Lipofectamine and 32 μg of MCH-R plasmid DNA.

Binding of [^{125}I]-MCH is measured using crude membranes prepared from HEK-293 or COS-7 cells transfected with MCH-R expression plasmids. Crude cell membranes from COS-7 or HEK-293 transfectants are prepared on ice, 48 hours post-transfection. Each 60 mm dish is washed twice with 3 ml of PBS, once with 1 ml homogenization buffer (50 mM Tris-HCl [pH 7.4], 5 mM $MgCl_2$, 2.5 mM EDTA, 30 μg/ml bacitracin, 1 μM phosphoramidon, 0.2 % BSA). 0.5 ml of homogenization buffer is added to each dish, cells are removed by scraping and then homogenized using a Polytron device (Brinkmann, Syosset, NY; 3 bursts of 10 sec. at setting 4). The homogenate is then centrifuged for 20 minutes at 11,000 x g at 0°C and the resulting crude membrane pellet (chiefly containing cell membranes and nuclei) is resuspended in homogenization buffer supplemented with 0.06% BSA (0.1 ml/60 mm dish) and kept on ice.

Binding reactions are performed at 20°C for 1 hour in a total volume of 0.5 ml containing: 0.1 ml of membrane suspension, 10 μl of [^{125}I]-MCH (0.05 to 1 nM; specific activity approximately 2000 Ci/mmol), 10 μl of competing compound(s) and 380-390 μl of homogenization buffer. Bound radioligand is separated by rapid vacuum filtration (Brandel 48-well cell harvester) through GF/C filters pretreated for 1 hour with 0.5% polyethylenimine. After application of the membrane suspension to the filter, the filters are washed 3 times with 3 ml each of ice cold 50 mM Tris-HCl [pH 7.4], 10 mM $MgCl_2$, 2.5 mM EDTA and 0.015% Triton X-100, and the bound radioactivity on the filers is quantitated by gamma counting. Specific binding (> 90%

of total) is defined as the difference between total binding and non-specific binding conducted in the presence of 100 nM unlabeled MCH.

  Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made

5 without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1.   A purified nucleic acid comprising a nucleotide sequence encoding for at least 5 contiguous amino acids of SEQ. ID. NO. 8.

2.   The nucleic acid of claim 1, wherein said nucleotide sequence encodes for amino acids 1-5 of SEQ. ID. NO. 8.

3.   The nucleic acid of claim 1, wherein said nucleotide sequence encodes for at least 9 contiguous amino acids of SEQ. ID. NO. 8.

4.   The nucleic acid of claim 1, wherein said nucleic acid comprises at least about 18 contiguous nucleotides of SEQ. ID. NO. 7.

5.   The nucleic acid of claim 1, wherein said nucleic acid comprises the nucleotide sequence of SEQ. ID. NO. 3.

6.   The nucleic acid of claim 1, wherein said nucleotide sequence encodes for the amino acid sequence of SEQ. ID. NO. 4.

7.   The nucleic acid of claim 1, wherein said nucleic acid comprises the nucleotide sequence of SEQ. ID. NO. 5.

8.   The nucleic acid of claim 1, wherein said nucleotide sequence encodes for the amino acid sequence of SEQ. ID. NO. 6.

9.   An expression vector comprising a nucleotide sequence encoding for a polypeptide comprising at least 5 contiguous amino acids of SEQ. ID. NO. 8.

10.   The expression vector of claim 9, wherein said nucleotide sequence is functionally coupled to an exogenous promoter.

11.   The expression vector of claim 10, wherein said nucleotide sequence encodes for at least 9 contiguous amino acids of SEQ. ID. NO. 8.

12.   The expression vector of claim 10, wherein said expression vector comprises the nucleotide sequence of SEQ. ID. NO. 3.

5   13.   The expression vector of claim 10, wherein said nucleotide sequence encodes the amino acid sequence of SEQ. ID. NO. 4.

14.   The expression vector of claim 10, wherein said expression vector comprises the nucleotide sequence of SEQ. ID. NO. 5.

10

15.   The expression vector of claim 10, wherein said nucleotide sequence encodes the amino acid sequence of SEQ. ID. NO. 6.

16.   A recombinant cell comprising the expression vector of claim
15   9.

17.   A method of preparing a MCH receptor polypeptide comprising the step of growing the recombinant cell of claim 16 under conditions wherein said polypeptide is expressed from said expression vector.

20

18.   A purified nucleic acid comprising a region of 20 contiguous nucleotides, wherein at least 16 nucleotides present in said region hybridize to a complementary region of 20 contiguous nucleotides present in SEQ. ID. NO. 7 or the complement thereof.

25

19.   A polypeptide comprising an amino acid sequence encoding for at least about 9 contiguous amino acids of SEQ. ID. NO. 8, wherein said polypeptide is substantially free of associated proteins.

30   20.   The polypeptide of claim 19, wherein said polypeptide comprises the amino acid sequence of SEQ. ID. NO. 4.

21.   The polypeptide of claim 19, wherein said polypeptide comprises the nucleotide sequence of SEQ. ID. NO. 6.

35

22.   A method for screening for a compound able to bind a MCH receptor comprising the steps of:

(a)   expressing a polypeptide comprising the amino acid sequence of SEQ. ID. NO. 4, SEQ. ID. NO. 6, or a fragment thereof, from recombinant nucleic acid, provided that said fragment comprises at least about 9 contiguous amino acids of SEQ. ID. NO. 8;

(b)   providing to said polypeptide a test preparation comprising one or more test compounds; and

(c)   measuring the ability of said test preparation to bind to said polypeptide.

23.   The method of claim 22, wherein said steps (b) and (c) are performed *in vitro*.

24.   The method of claim 22, wherein said steps (a), (b) and (c) are performed using a whole cell.

25.   The method of claim 22, wherein said polypeptide is expressed from an expression vector.

26.   The method of claim 25, wherein said polypeptide comprises the amino acid sequence of SEQ. ID. NO. 6.

27.   The method of claim 25, wherein said step (b) further comprises the presence of labeled MCH, and said step (c) measures the ability of said test preparation to inhibit binding of said labeled MCH to said polypeptide.

28.   A method for screening for a compound able to modulate MCH receptor activity comprising the steps of:

(a)   contacting a cell line expressing recombinant nucleic acid encoding for a MCH receptor comprising the amino acid sequence of SEQ. ID. NO. 4 or 6 with a test preparation comprising one or more test compounds; and

(b)   measuring the effect of said test preparation on the activity of said receptor.

29.   The method of claim 28, wherein said MCH receptor comprises the amino acid sequence of SEQ. ID. NO. 4.

30.   The method of claim 28, wherein said MCH receptor consists

5   of the amino acid sequence of SEQ. ID. NO. 6.

31.   The method of claim 30, wherein said method further comprises the presence of an MCH receptor agonist.

10   32.   A method for suppressing appetite comprising the step of administering to a patient an effective amount of means for decreasing MCH receptor expression targeting a nucleic acid region within SEQ. ID. NO. 7.

33.   The method of claim 32, wherein said means is enzymatic

15   nucleic acid or antisense nucleic acid.

SEQUENCE LISTING

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C12N 5/10, 15/12, 15/63
US CL :435/69.1, 320.1, 325; 536/23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/69.1, 320.1, 325; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BRS, STN, MEDLINE, PIR, GENEMBL GENESEQ, SPTREMBL

search terms: melanin concentrating hormone, receptor, MCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - A	EP 0 848 060 A2 (SMITHKLINE BEECHAM CORPORATION) 17 June 1998 (17.06.1998), page 7 and figures 1-2.	1-4 ------- 5-18

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 OCTOBER 2000	27 OCT 2000

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer MICHAEL PAK
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such
   an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

   Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable
   claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers
   only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1-18

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

                       ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)*

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-18, drawn to a nucleic acid, an expression vector, a recombinant cell, and a method of preparing the MCH receptor.

Group II, claim(s) 19-21, drawn to a polypeptide.

Group III, claim(s) 22-31, drawn to a method for screening a compound.

Group IV, claim(s) 32-33, drawn to a method for suppressing appetite.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features because the product of claims 1-3 is anticipated by SMITHKLINE BEECHAM CORPORATION (EP 0848060 A2, 17 June 1998) and thus, does not share a special technical feature with any other group.

The special technical feature of Group I is a nucleic acid, an expression vector, a recombinant cell, and a method of preparing the MCH receptor. Pursuant to 37 CFR 1.475(d), these claims are considered by the ISA/US to constitute the main invention, and none of the related groups II-IV correspond to the main invention.

The products of Group II does not share the same or corresponding special technical feature with Group I, because they are drawn to products having materially different structures and functions, and each defines a separate invention over the art.

The methods of Groups III-IV, do not share the same or corresponding special technical feature with Group I, because the methods have materially different process steps and are practiced for materially different purposes, and each defines a separate invention over the art.

Since Groups I-IV do not share a special technical feature, unity of invention is lacking.